

Humoral and T cell responses to SARS-CoV-2 vaccine booster and anti-SARS-CoV-2 monoclonal antibodies in patients with End Stage Kidney Disease

Supplementary Methods

Study population

Our prospective study included 42 consecutive consenting adult kidney transplant recipients and 10 patients on hemodialysis at the Nephrology, Dialysis and Transplantation Unit, University of Foggia, Foggia, Italy who had received 3 COVID-19 mRNA vaccine doses. During the month prior study entry, a subset of 10 kidney transplant patients experienced a breakthrough infection (confirmed by PCR) and received no further treatment, while 12 patients received a 4th mRNA vaccine dose. The remaining 20 kidney transplant recipients and the 10 patients on hemodialysis received a single dose of Evusheld (150mg tixagevimab + 150mg cilgavimab; AstraZeneca). At study entry, all patients had a negative PCR swab test for SARS-CoV2. Blood samples were collected at the time of study entry (on the day of the 4th vaccine dose or Evusheld), and at 3 and 6 months thereafter. Written consent was obtained from all participants included in the study.

Anti-SARS-CoV-2 Antibody Measurement. Detection of SARS-CoV-2 specific IgG antibodies directed against the full trimeric spike protein, the individual spike 1 (S1), spike 2 (S2), and receptor binding domains (RBD) of the spike protein, and the nucleocapsid protein (NC) and Spike S1 fragments from six other coronaviruses, namely HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, MERS-CoV and SARS-CoV-1 was performed with the One Lambda single-antigen bead assay [LABScreen™ COVID Plus®, One Lambda], as previously described.^{S1} Plates were then analyzed on a Luminex FLEXMAP 3D instrument (Luminex Corp. Austin, TX). Thresholds for positivity of each antibody were defined based on package insert.

IFN- γ FluoroSpot. PBMC were seeded at 500,000 cells/well in 96 well FluoroSpot plates (Cellular Technology Ltd) with CTL-Test Media cell culture medium containing 1% L-glutamine and anti-CD28 mAb (0.1 μ g/ml). Test wells were performed in duplicate and supplemented with 15-mer overlapping peptides covering the immunedominant regions of the S glycoprotein (573 amino acids) (PepTivator SARS-CoV-2 Prot S, Miltenyi Biotec), the complete NC protein (102 peptides) (PepTivator SARS-CoV-2 Prot N, Miltenyi Biotec) and the complete M protein (53 peptides) (PepTivator SARS-CoV-2 Prot M, Miltenyi Biotec) at a final concentration of 0.5 μ g/ml. Negative control wells contained 20% DMSO and lacked peptides while positive control wells included CEF-MHC Class I Peptide Pool “Plus”. The fluorophore conjugates used were CTL Red-690 and FITC-520. Assays were incubated for 24 h at 37°C. The readout was performed following the manufacturer’s instructions for the Human IFN- γ FluoroSpot kit and spots were counted using an automated ImmunoSpot Analyzer Professional System (both from Cellular Technology Ltd.). To quantify antigen-specific responses, spots of the negative control wells were subtracted from the mean spots test wells, and the results were expressed as IFN- γ spot forming units (SFUs) per 5×10^5 PBMCs.

Statistical analysis

Graphs and statistics were completed in GraphPad Prism (GraphPad Software, La Jolla, CA). We expressed results as means and SDs. Comparison across values at different time points was computed using two-way ANOVA. *P* values were computed to assess significance of individual comparisons, with a value of *P*=0.05 considered as statistically significant.

SUPPLEMENTARY REFERENCE

S1. Bray RA, Lee JH, Brescia P et al. Development and Validation of a Multiplex, Bead-based Assay to Detect Antibodies Directed Against SARS-CoV-2 Proteins. *Transplantation*. 2021 Jan 1;105(1):79-89.

Table S1. Clinical characteristics.

	4th Dose (n=12)	COVID (n=10)	Evusheld (KTx) (n=20)	Evusheld (HD) (n=10)
Male:Female, n	9:3	7:3	14:6	9:1
Age (<i>years</i>)	56.8 ± 11.8	53.2 ± 10.5	57.95 ± 10.5	64.6±12.2
Comorbidities				
Hypertension (%)	90%	90%	80%	70%
Diabetes (%)	25%	20%	30%	60%
Cardiovascular Disease (%)	16.6%	30%	50%	40%
Time since transplant (<i>mo</i>)	140 (90-155)	105 (87-133)	109 (93-125)	-
Serum creatinine (<i>mg/dl</i>)	1.8 ± 0.8	1.7 ± 0.3	1.6 ± 0.6	-
Proteinuria (<i>g/24h</i>)	0.5 ± 0.4	0.3 ± 0.3	0.4 ± 0.9	-
Immunosuppression				
Steroids, n	11	10	19	
MMF/MPA, n	12	10	20	
CNI, n	12	10	20	

Data are expressed as n, %, mean ± SD, or median (IQR). CNI: calcineurin inhibitor; MMF: Mycophenolate mofetil; MPA: Mycophenolic acid.